Short Communication

Uptake and Phytoaccumulation of Chromium at Seedling Stage in Some Selected Tree Species

Mehari Alebachew[1*], Hameed Sulaiman[2] and Negussie Retta[3]

[1]EIAR, Holetta Agricultural Research Center, P O Box 2003, Addis Ababa, Ethiopia
[2]Addis Ababa University, Environmental Science Program, P O Box 1176, Addis Ababa, Ethiopia
[3]Addis Ababa University, Department of Chemistry, P O Box 1176, Addis Ababa, Ethiopia

Abstract: The objective of the study was to identify shoot and root uptake of chromium (Cr) for screening tree species and recommend the best Cr accumulator. Both trivalent and hexavalent Cr species at four concentration levels 0 (control), 10, 100 and 1000 ppm were used as treatments. The growing medium was prepared by mixing 3:3:1:1, forest soil, local soil, manure, and sand, respectively. The parts of *Ricinus communis* showed a very good Cr storage capacity followed by *Millittia ferruginea* and *Eucalyptus camaldulensis*. The parts accumulate nearly the same amount of Cr in all the species. With regard to the uptake of Cr, *Ricinus communis* was the most efficient in its accumulation capacity as it was found in this study to contain with the highest level of Cr per gram of dry matter.

Keywords: Chromium; *Eucalyptus camaldulensis; Millittia ferruginea; Ricinus communis*

1. Introduction

Chromium is one of the major heavy metals discharged from tanneries. Unlike organic compounds, the heavy metal chromium cannot be degraded and the clean up technology requires removal. The removing process employs physiochemical agents, which can dramatically inhibit soil fertility with subsequent negative impacts on the ecosystem. The EPA (1997) of Ethiopia found that, of the existing industries in the country, only very few have the treatment systems. The rest discharge their effluents into the water bodies and land without proper treatment. This had resulted in serious water and soil pollution in the area (EPA, 1997). However, no initiative has been undertaken to address and remediate the hazard. Phytoremediation is an emerging technology that can be considered for solving the problem of contaminated sites because of its cost effectiveness, aesthetic advantages and long-term applicability (Cunningham, 1996). Many research findings have indicated that plants have the genetic potential to remove many toxic heavy metals from the soil (Arun *et al*., 2005). This study was initiated with the objective of investigating the phytoremediation potential of three commonly grown tree species (*Millittia ferruginea*, *Ricinus communis* and *Eucalyptus camaldulensis*) in Ethiopia at their seedling stage. The selected tree species have a number of advantages, such as economic, environmental and social benefits. Research results in different countries showed that these species have the capacity to remediate Cr contaminated sites. (Cesaer *et al*., 2005).

2. Material and Methods

The research was carried out at Holetta Agricultural Research Center, Ethiopia. The center is located 28 km west of Addis Ababa, with an altitude of 2400 m above sea level. Geographically, the center is located between 9^o 06` N latitude and 38$^o$ 08` E longitude. The climate of the center is sub-humid with an average annual rainfall of 1100 mm and with average maximum and minimum temperatures of 21 ºC and 6 ^{o}C, respectively. The experiment was carried out in the greenhouse of the center.

2.1. Treatments and Experimental Design

The treatments were factorial combination of 3 tree species and 4 levels of two Cr species each. Trivalent Cr was applied as $Cr_2 (SO_4)_3.6H_2O$ and Cr (VI) as $K_2Cr_2O_7$ with 10,100 and 1000 ppm and control i.e. without Cr (0 ppm). The experiment was conducted in completely randomized design (CRD) with 3 replications.

2.2. Media Preparation and Collection of Plant and Soil Samples

Forest soil, local soil, manure and sand required for potting media preparation were collected and placed in pretreated sites before mixed. The soil media were sieved and then mixed with a proportion of 3:3:1:1 with forest soil, local soil, sand and manure, respectively, then put into 16 cm diameter x 20 cm depth polythene bags and transferred to the greenhouse. The initial soil samples were analyzed for Cr and subsequently after the experiment for final Cr analysis. The inner four best seedlings were assessed for data collection after three months of sowing. Seedlings were uprooted, preserved in polythene bags and finally taken to the laboratory for plant Cr analysis.

2.3. Chromium Extraction in the Soil Media and Plant Parts

For Cr extraction in the soil media, 10 ml of concentrated HNO_3 was added to 0.5 g of soil samples in a 50 ml digestion tube and allowed to stand overnight at room temperature. The digestion tubes were placed in a heating block for one hour at 150 ºC, tubes were then allowed to cool for one hour and 2 ml of 30% H_2O_2 was added. The contents were mixed by swirling and then heated at 150 ºC until digestion was completed. Digested soil samples were analyzed for Cr in AAS (Atomic Absorption

*Corresponding author. E-mail: mehariatesfaye@yahoo.com

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Spectrophotometer -Varian Spectra AA-220) with air acetylene flame at 358 ηm and with 0.2 spectral slit widths.

For Cr extraction in plant parts, 5 ml of concentrated HNO_3 was added to 0.25 g of dried plant sample in a 50 ml digestion tube and allowed to stand overnight at room temprature.The digestion tubes were placed in a heating block for four hours at 150 ºC. Color change was observed during digestion from orange to pale yellow and until it become colorless. Digested plant samples were also analyzed for Cr in AAS similar to that of soil samples. Both the soil and plant Cr extraction procedures used were modified from (Arun *et al*., 2005).

3. Results

3.1. Chromium Concentration in the Soil Media

The total Chromium (Cr tot) concentration in the soils treated with two Cr species was not significantly different from each other. The Cr (tot) concentration in Cr (VI) (62.28 μg g^{-1} dry soil) treated soil was lower than Cr (III) (67.43 μg g^{-1} dry soil) treated soil (Table 1). However, it varied significantly in soils grown with three tree seedlings after harvest. The highest Cr (tot) value in the soil was recorded for *Eucalyptus camaldulensis* (76.26 μg g^{-1} dry soil) followed by *Millittia ferruginea* (68.53 μg g^{-1} dry soil) and *Ricinus communis* (49.77 μg g^{-1} dry soil) (Table 1). Nevertheless, the Cr (tot) level in the soil was linearly correlated to the Cr application with total Cr of 35.35, 58.15, 71.62, 94.29 μg g^{-1} of dry soil for different soil doses of 0 ppm, 10 ppm, 100 ppm and 1000 ppm respectively (Table 1). The highest concentration of Cr (tot) in hexavalent Cr treated soil was recorded in the 1000 ppm soil dose of *Eucalyptus camaldulensis* (98.71 μg g^{-1} dry soil) and *Ricinus communis* (96 μg g^{-1} dry soil). However in the same treatment the lowest value of Cr (tot) was recorded in the control of *Millittia ferruginea* (31.20 μg g^{-1} dry soil) followed by 10 ppm soil dose (31.28 μg g^{-1} dry soil) of the same tree species. In addition, the highest Cr (tot) in soil treated with trivalent Cr was recorded in the 1000 ppm soil dose of *Eucalyptus camaldulensis* (113.47μg g^{-1} dry soil) and *Ricinus communis (*108.93 μg g^{-1} dry soil). However, the lowest value of Cr (tot) concentration was recorded in the control of *Millittia ferruginea (*28.47μg g^{-1} dry soil) and *Eucalyptus camaldulensis (*32.17 μg g^{-1} dry soil) for trivalent Cr treated soil.

Table 1. Total Cr (μg g-1 of dry soil) in soil.

Treatment	(μg g^{-1} of dry soil)	Treatment	(μg g^{-1} of dry soil)
EC control	36.75[cd]	MF Cr (VI) control	31.20[cd]
EC Cr (III) 10 ppm	69.65[bc]	MF Cr (VI) 10 ppm	31.28[cd]
EC Cr (III) 100 ppm	81.73[bc]	MF Cr (VI) 100 ppm	47.65[c]
EC Cr (III) 1000 ppm	113.47[a]	MFCr (V I) 1000 ppm	70.84[bc]
EC Cr (VI) 10 ppm	83.26[b]	RC control	39.50[cd]
EC Cr (VI) 100 ppm	89.77[ab]	RC Cr (III) 10 ppm	60.98[bc]
EC Cr (VI)1000 ppm	98.71[ab]	RC Cr(III)100 ppm	85.60[b]
MF control	29.84[d]	RC Cr(III) 1000 ppm	108.93[ab]
MF Cr (III) 10 ppm	47.73[c]	RC Cr (VI) 10 ppm	55.97[c]
MF Cr (III) 100 ppm	63.24[bc]	RC Cr (VI) 100 ppm	61.73[b]
MF Cr (III) 1000 ppm	77.73[bc]	RC Cr(VI)1000 ppm	96.04[ab]

Letters with the same superscript are not statistically significant at (P ≤ 0.05)
Cr = chromium; Cr (III) = Trivalent chromium; Cr (VI) = hexavalent chromium; EC = Eucalyptus camaldulensis; μg = microgram; MF = Millittia ferruginea; ppm = parts per million; RC = Ricinus communis; SE = standard error; Tot = total

3.2. Chromium Accumulation in shoots and roots of tree species

A glance at Table 2 clearly shows a significant difference in the uptake of Cr (tot) out of all treatments of *Ricinus communis*. The maximum Cr (tot) soil accumulation was in *Ricinus communis* (145.34 μg g^{-1}DM) followed by *Millittia ferruginea* (81.01 μg g^{-1}DM) and *Eucalyptus camaldulensis* (55.6 μg g^{-1}DM). Out of the two Cr species treated soil, most of the tree species accumulated more from Cr (III) treated soil than Cr (VI) except *Ricinus communis*.

There were no significant differences in the uptake of Cr (tot) for *Eucalyptus camaldulensis* and *Ricinus communis*. However, significant differences were observed in the uptake of Cr (tot) among the treatments of *Millittia ferruginea*. The maximum Cr (tot) soil accumulation of the root was attained for *Ricinus communis* (152.08 μg g^{-1}DM) followed by *Millittia ferruginea* (85.11μg g^{-1}DM) and *Eucalyptus camaldulensis* (42.70 μg g^{-1}DM) (Table 2). Out of the two Cr species treated soil, most of the tree species accumulated more from Cr (III) treated soil in the root than Cr (VI) treated soil except *Ricinus communis* as of the shoots. The highest Cr(tot) accumulation in the roots was recorded in *Ricinus communis* of hexavalent Cr (207.99 μg g^{-1}DM) followed b*y Millittia ferruginea* of trivalent Cr (115.74 μg g^{-1}DM*)* an*d Eucalyptus camaldulensis* of trivalent Cr (80.84 μg g^{-1}DM) (Table 2). However, the lowest value of Cr (tot) accumulation of the two Cr species was recorded in *Eucalyptus camaldulensis* of hexavalent Cr (12.51 μg g^{-1}DM) followed by *Millittia ferruginea* of the same Cr species (54.48 μg g^{-1}DM) and *Ricinus communis* of trivalent Cr species (96.18 μg g^{-1}DM) (Table 2).

Table 2. The average chromium concentration (μg g $^{-1}$ of dry matter) of three tree species (plant parts).

Treatment	*Eucalyptus camaldulensis*		*Millittia ferruginea*		*Ricinus communis*	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Cr (III) 10 ppm	42.63^{b}	35.93^{b}	82.93^{b}	72.84^{b}	71.68^{bc}	45.84^{c}
Cr (III)100 ppm	1 14.89^{ab}	90.31^{ab}	130.16^{ab}	130.11^{ab}	143.73^{b}	113.96^{bc}
Cr (III)1000 ppm	158.20^{a}	163.60^{a}	199.01^{a}	181.11^{a}	150.09^{b}	158^{b}
Cr (VI)10 ppm	16.49^{bc}	17.60^{b}	27.88^{b}	33.41^{b}	242.98^{a}	236.52^{ab}
Cr (VI)100 ppm	19.33^{b}	22.27^{b}	43.64^{b}	78.24^{b}	257.71^{a}	248.17^{a}
Cr (VI)1000 ppm	*	*	124.13^{ab}	118.67^{ab}	300.4^{a}	309.91^{a}
Control	4.68^{c}	3.94^{b}	36.57^{b}	16.94^{b}	25.04^{c}	25.30c
Mean	42.70	55.60	85.11	81.01	152.08	145.34
SE±	30.91	30.91	30.91	30.91	92	30.92

**- The seedlings died in this treatment*

Letters with the same superscript in the column are not statistically significant at (P ≤ 0.05)

3.3. Chromium Accumulation Factor (CAF) of the tree species

The Cr accumulation factor (CAF) is the ratio of Cr accumulated in plant parts (shoots and roots) divided by Cr content in the soil media. With regard to the plant parts analyzed, it was seen that the roots and shoots had CAF for all three tree species with corresponding values of (0.78 Vs 0.74, 1.50 Vs 1.88 and 2.12 Vs 2.22) of *Eucalyptus camaldulensis*, *Millittia ferruginea* and *Ricinus communis* respectively (Table 3). This is due to similar retention of Cr in the roots and the shoot parts of the tree species. CAF varied significantly among the shoot and root parts of the three tree species. The general pattern of CAF was highest in *Ricinus communis* (2.17) followed by *Millittia ferruginea* (1.80) and the lowest CAF was seen in *Eucalyptus camaldulensis* (0.21) (Table 3). CAF in trivalent chromium was higher than hexavalent chromium treated soil in all the tested tree species except *Ricinus communis*. Out of all tree species, the highest value of CAF was recorded in hexavalent Cr treated soil of *Ricinus communis* (3.11) followed by trivalent Cr species of *Millittia ferruginea* (2.00) and the lowest CAF was recorded in *Eucalyptus camaldulensis* (0.24) of hexavalent Cr (Table 3).

Table 3. Effect of chromium speciation and Cr levels on Cr accumulation factor (CAF).

Treatment	*Eucalyptus camaldulensis*		*Millittia ferruginea*		*Ricinus communis*	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Cr (III)10 ppm	0.75^{a}	0.75^{a}	1.63^{ab}	1.43^{b}	1.25^{b}	0.88^{b}
Cr (III)100 ppm	1.49^{a}	1.20^{a}	2.07^{ab}	2.07^{ab}	1.81^{b}	1.49^{b}
Cr (III)1000 ppm	1.76^{a}	1.76^{a}	3.21^{a}	3.10^{a}	1.43^{b}	1.45^{b}
Cr (VI)10 ppm	0.19^{a}	0.25^{a}	1.56^{ab}	1.77^{ab}	4.47^{a}	4.35^{a}
Cr (VI)100 ppm	0.22^{a}	0.27^{a}	0.91^{b}	1.73^{b}	4.27^{a}	4.10^{a}
Cr (VI)1000 ppm	*	*	1.67^{ab}	1.81^{ab}	3.13^{ab}	3.23^{b}
Control	0.27^{a}	0.21^{a}	1.99^{ab}	0.92b	0.69^{b}	0.74^{b}
Mean	0.78	0.74	1.88	1.50	2.22	2.12
SE±	0.69	0.69	0.69	0.69	0.69	0.69

**- The seedlings died in this treatment*

Letters with the same superscript in the column are not statistically significant at (P ≤ 0.05)

4. Discussion

Cr (tot) accumulation was highest in *Ricinus communis* (148.73 μg g^{-1}DM) followed by *Millittia ferruginea* (80.07 μg g^{-1}DM) and the lowest accumulation of Cr was in *Eucalyptus camaldulensis*. *Ricinus communis* accumulated more of Cr (tot) (412.61 μg g^{-1}DM) in hexavalent Cr treated soil than in trivalent treated soil (182.31 μg g^{-1}DM). However, *Eucalyptus camaldulensis* accumulated 154.78 μg g^{-1}DM of Cr (tot) in trivalent treated soil and 26.45 μg g^{-1}DM of Cr (tot) in Cr (VI) treated soil. Furthermore, *Millittia ferruginea* accumulated 217.93 μg g^{-1}DM and 114.36 μg g^{-1}DM of Cr (tot) in Cr (III) and Cr (VI) treated soil, respectively. The highest uptake of Cr was seen in *Ricinus communis* treatment. Caeser *et al.* (2005) reported in a study conducted on soil polluted by nickel that the Ricinus was very efficient in the removal of Ni compared to other plants. It exhibited a Cr content of 309.91, 300.4, 257, and 248 μg g^{-1} DM at 1000 ppm of for the shoots, roots, and 100 ppm for the shoots and roots respectively. On another study in *Eucalyptus,* Grant *et al.* (2002) found that Cr was remediated by using *Eucalyptus camaldulensis* species. In general, roots accumulated nearly similar Cr content to the shoots in all the tree seedlings. However, *Millittia ferruginea* shoots accumulated more of Cr (tot) in Cr (VI) treated soil than roots. The CAF also showed a similar trend to the chromium accumulated in all the tree seedlings.

The reason for the high accumulation of Cr in both shoots and roots parts of the plant could be because of transformation of Cr (VI) to Cr (III) in the root environment and changed into non toxic form of Cr (III) then translocated into the shoots. The Cr (VI) in cells is probably readily reduced to Cr (III) and the Cr (III) is retained in the root cortex cells (Zayed *et al.,* 1998). The accumulation factor for both Cr species derived in the present study is in agreement with the reports of Khan (2001). This could be because in the uptake pattern of Cr from soil, which varies from species to species and within the species, the concentration also differs between different parts of the plant (Pulford *et al*., 2001). The evidence of transfer of Cr from root to shoot implies that the prospect of using these trees as their phytoremediation potential in Cr-contaminated site is high. *Ricinus communis* is a potential Cr accumulator followed by *Millittia ferruginea* and, therefore, it can be recommended in areas where the Cr contamination levels are moderate to high. However, *Eucalyptus camaldulensis* can also be used as a potential species where Cr contamination levels are low to moderate. Furthermore, the *Ricinus communis* can be utilized in other research for phytoremediation.

5. Conclusion

The total chromium concentration was found to be higher in trivalent Cr treated soil when compared to hexavalent Cr treated soil. The dosage of Cr in soil had a positive correlation with the measured amount of total Cr concentration. When it comes to the uptake of total Cr in general, *Ricinus communis* accumulated the highest amount of total Cr followed by *Millittia ferruginea* and *Eucalyptus camaldulensis*. This general trend was confirmed further by the actual quantification of chromium accumulation factor which showed that the CAF of *Ricinus communis* is comparatively higher than the other two species. The CAF variations with respect to Cr speciation revealed that, in all the tested tree species the CAF recorded in trivalent Cr treated soil was higher than hexavalent Cr treated soil except *Ricinus communis.* This indicates the tolerance of *Ricinus communis* to the toxicity of hexavalent chromium. The study recommends, of the three candidate species for phytoremediation of Chromium contaminated sites, *Ricinus communis* has the potential for remediating the tannery-contaminated soil with high amounts of Cr followed by *Millittia ferruginea*. However, *Eucalyptus camaldulensis* is not the suitable species since it is found to be sensitive to high levels of Cr especially the hexavalent species which in this study failed to survive and could be tried in low to moderate Cr contaminated soils.

6. References

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